

REMARKS

Claims 1-22 are pending in the subject application. The Examiner has withdrawn claims 8-22 from consideration as being drawn to a non-elected species. Claims 1-7 have been examined on the merits. Claims 1, 6 and 7 have been amended to more particularly point out and clearly claim the subject matter the Applicants consider their invention.

Support for the amendments may be found throughout the application but particularly on p. 9, lines 17-25, p. 10, lines 1-9, p. 12, lines 11-24, p. 24, lines 12-22, p. 26, lines 13-16, p. 41, lines 4-17, and p. 43, line 20 to p. 44, line 19.

The Examiner has objected to various informalities in the claims and specification. Appropriate correction is made herewith.

1. Rejections under 35 U.S.C. § 112

1.1. Rejection of claims 1-7 under 35 U.S.C. § 112, Second Paragraph Indefiniteness

The Examiner rejects claim 1 as vague and indefinite because it is allegedly unclear as to the use of the terms MAD2 and TUB1 as a sole means of identifying the genes recited in the claims.

Applicants respectfully disagree and assert that the claim recitations “MAD2” and “TUB1” are clear on their face and assert that the Examiner has provided no evidence or basis for the statement that different labs refer to these genes by different designations.

The recitation “TUB1”, refers to a gene that encodes the ubiquitous protein alpha-tubulin. See p. 40, lines 35-26. A simple search of the iHOP (Information Hyperlinked Over Proteins; <http://www.pdg.cnb.uam.es/UniPub/iHOP/>; see Hoffmann, R., Valencia, A. A Gene Network for Navigating the Literature. *Nature Genetics* 36, 664 (2004)) database with the term “TUB1” yields a range of accession numbers for the TUB1 gene and protein sequences: UniProt P09733, NCBI Gene 854889, NCBI RefSeq NP_013625.1, NCBI Accession AAA35180 and CAA86653, see Exhibit A. Moreover, the search yields a long list of scientific publications describing the structural and functional attributes of TUB1. Finally, the search identifies and hyperlinks to similar information on TUB1 homologs in humans, mouse, *Drosophila*, *C.elegans* and *Arabidopsis*.

Additionally, one of skill in the art would recognize that “MAD2” refers to a yeast mitotic spindle checkpoint component gene. A simple search of the iHOP database with the term “MAD2” yields a range of accession numbers for the MAD2 gene and protein sequences: UniProt P40958; NCBI Gene 853422; NCBI RefSeq NP_012504.1; NCBI Accession AAA21385, CAA89321 see Exhibit B. Moreover, the search yields a long list of scientific publications describing the structural and functional attributes of MAD2. Finally, the search identifies and hyperlinks to similar information on MAD2 homologs in humans (Exhibit B, Paper No. 1) , mouse (Exhibit B, Paper No. 2), *Drosophila* (Exhibit B, Paper No. 3), *C.elegans* (Exhibit B, Paper No. 4) and *Arabidopsis* (Exhibit B, Paper No. 5).

In view of this evidence documenting that one of skill in the art would readily understand what the terms “TUB1” and MAD2” refer to Applicants respectfully request withdrawal of this rejection.

The Examiner rejects claims 1-2, and 7 as being indefinite for allegedly failing to recite a control step. Applicants respectfully assert that the amendments to claim 1 fully addresses this ground for rejection.

1.2. Rejection of claims 1-7 under 35 U.S.C. § 112, First Paragraph, Written Description

The Examiner rejects claims 1-7 under 35 U.S.C. § 112, first paragraph, for allegedly encompassing subject matter which was not adequately described in the application. The Examiner asserts that the claim recitation “mutated MAD2” including analogs and homologs thereof, generically covers a large number of species. However, the Examiner alleges that the disclosure only exemplifies one yeast MAD2 mutant and that no structural information is provided to define the genus of molecules. Applicants respectfully disagree.

With regard to “homologs,” the Examiner has pointed out that the term has been defined in the specification (Office Action of 10/21/04, p. 5, lines 6-9). Furthermore, one of skill in the art would recognize homologs to be structurally similar genes and/or proteins that are part of a family whose members are conserved across a range of species. For example, the MAD family contains, but is not limited to, Yeast MAD2, *Xenopus* X MAD, Human hsMAD, and *Drosophila* MAD. Moreover, a standard definition of “homology” in the context of molecular biology refers to “[s]imilarity in structure of ... a molecule, reflecting a common evolutionary origin. Specifically, such similarity in protein or nucleic acid sequence.” Molecular Biology of the Cell,

3rd Edition, 1994, p. G-12 (attached as Exhibit C). Additionally, the iHOP search referred to in 1.1. *supra*, readily identifies MAD2 homologs (see link “Homologues of MAD2”; Ex. B) in humans (Exhibit B, Paper No. 1), mouse (Exhibit B, Paper No. 2), *Drosophila* (Exhibit B, Paper No. 3), *C.elegans* (Exhibit B, Paper No. 4) and *Arabidopsis* (Exhibit B, Paper No. 5).

Moreover, Figure 1A of Li *et al.*, Science 1996, 274, 5285; 246-248 (of record) shows an amino acid sequence alignment of human, *Xenopus* and yeast MAD2 proteins. The alignment is further solid evidence that a person of ordinary skill in the art would readily understand the structural features of the MAD family of genes and know what constitutes a MAD2 homolog and what does not.

Additionally, “mutated MAD2 gene” refers to a variety of known and unknown mutant MAD2 alleles as well as mutants of MAD2 homologs in other species. The skilled artisan would have known that a mutated MAD2 gene could be the result of nucleic acid deletions, insertions or substitutions upstream, downstream, within or in between the MAD2 genomic coding regions. As such, it would not only be highly impractical to describe MAD2 mutants by way of a specific sequence as suggested by the Examiner; it would also be unnecessary because one of skill in the art would know to identify mutated MAD2 homologs because of the well described nature of the gene itself across a range of species.

Applicants have amended the claims to remove the term “analogs” of MAD2. Applicants respectfully assert that the amendments to claim 1 and the remarks above fully addresses this ground for rejection and withdrawal thereof is respectfully requested.

2. Rejection under 35 U.S.C. § 102

The Examiner rejects claims 1, 3-4 and 6-7 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,972,640 to Drubin *et al.* (hereinafter ‘Drubin’) The Examiner argues that Drubin teaches a method for identifying an anti-mitotic compound comprising the steps of contacting a cell with an agent, wherein the cell has a functionally disrupted mitotic check point, *e.g.*, having at least one mutation in a MAD2 or TUB1 gene; and detecting the sensitivity or the response of the cells to the agent.

Furthermore, the Examiner states that the anti-mitotic activity of any identified compound is then confirmed using anti-tubulin immunofluorescence to identify spindle defects. Applicants respectfully disagree.

However, in order to expedite prosecution, Applicants have amended claim 1 to recite a method of identifying a drug that inhibits growth or replication of a cell having a mutated MAD2 gene or homolog thereof, the method comprising the steps of identifying a secondary gene comprising providing a plurality of cells having a genome, which includes at least one mutated MAD2 gene or homolog thereof; effecting one or more mutations in the genome of the cells, at one or more secondary genes; selecting those cells having at least one additional mutation that proves lethal to the cells only when the mutated MAD2 gene is present; determining a site in the genome of the cells in which the at least one lethal mutation is located, to provide a secondary gene; contacting a cell having a mutated MAD2 gene or homolog thereof with a drug; and identifying the drug by determining whether the drug modulates the activity of the wildtype secondary gene such that the drug is lethal to the cell having a mutated MAD2 gene but not to a wild type cell.

As such, claim 1 has now been amended to include a step for first identifying a secondary gene which is synthetically lethal when mutated and present in combination with a mutated MAD2 gene before the steps of screening candidate drug compounds. Drubin merely teaches a screen for antimetabolic compounds that will selectively inhibit the growth of cells that are mutant in either a *mad* or *bub* gene. Applicants respectfully assert that Drubin does not teach identifying the claimed secondary genes. Additionally, Drubin does not teach identifying specific drugs by determining whether the drug modulates the activity of wildtype secondary genes such that the drugs are lethal to cells having a mutated MAD2 gene but not to a wild type cell.

Applicants respectfully submit that the foregoing amendments have fully addressed the Examiner's rejection and, therefore, request its removal.

3. Rejection under 35 U.S.C. § 103

The Examiner also rejects claims 1-7 under 35 U.S.C. § 103(a) as allegedly being unpatentably obvious over Drubin in view of Li *et al.*, Science 1996, 274, 5285; 246-248 (hereinafter 'Li'). The Examiner concedes that Drubin does not teach that the cells upon which the claimed methods may be performed can be tumor cells. However, Li allegedly teaches this connection.

Applicants respectfully submit that Drubin does not anticipate claims 1-7 for the reasons above because it does not teach the step of identifying secondary genes that are synthetically lethal

to MAD2 and selecting compounds by their ability to modulate wild type secondary genes. For the same reasons, Drubin does not render the claimed invention unpatentably obvious. For example, nothing Drubin teaches or suggests the identification of wild type genes which in mutant form are synthetically lethal in combination with mutant MAD2, let alone screening compounds that modulate such genes' wild type products. Moreover, Li contains no disclosure that overcomes the deficiencies of Drubin.

Applicants respectfully submit that the foregoing amendments and remarks have fully addressed the Examiner's rejections under 35 U.S.C. § 103 and, therefore, request their removal.

CONCLUSION

To the extent necessary, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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Symbol Name

MAD2L1 Mitotic spindle assembly checkpoint protein MAD2A

Synonyms

HsMAD2, HSMAD2, MAD2, MAD2-like 1, MAD2 mitotic arrest deficient-like 1 (yeast)

Organism

Homo sapiens

UniProt Q13257

IntAct Q13257

PDB Structure 1DUJ, 1KLQ

OMIM 601467

NCBI Gene 4085

NCBI RefSeq NP_002349

NCBI Accession AAC50781, AAC52060, AAH00356

Homologues of MAD2L1 ... **new**

More literature information for this gene  ...

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However, Bub1 has several **functions** that are not shared with Mad2.

Mad2 phosphorylation **regulates** its association with Mad1 and the APC/C.

In addition, in vitro cdk1-phosphorylated Cdc20 **interacts** with Mad2 rather than APC/ C.

When expressed in wing imaginal disks, hSmad2 **induced** oversize wings while hSmad3 induced cell death.

In this model, the unattached kinetochore is postulated to catalytically convert Mad2 to a form that **binds** Cdc20.

Mutagenesis and NMR titration experiments show that a C-terminal flexible region of Mad2 is required for **binding** to Cdc20.

Upon checkpoint activation, Mad2 **binds** directly to Cdc20 and inhibits the anaphase-promoting complex or cyclosome (APC/C).

Using deletion mutagenesis and peptide mapping, we have identified the sequences in Cdc20 that **target** it to Mad2 and the APC, respectively.


In the course of these studies, a truncation mutant of Cdc20 (1-153) that constitutively **binds** Mad2 but fails to bind the APC was identified.

These data suggest that the phosphorylation state of Mad2 **regulates** its checkpoint activity by modulating its association with Mad1 and the APC/C.

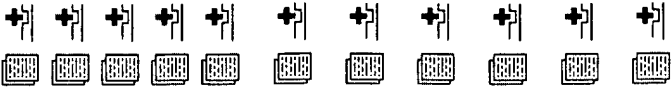
The spindle checkpoint protein Mad1 **recruits** Mad2 to unattached kinetochores and is essential for Mad2-

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Cdc20 complex formation in vivo but not in vitro.

We show that RNAi-mediated suppression of Mad1 function in mammalian cells causes loss of Mad2 kinetochore localization and impairment of the spindle checkpoint.

When the spindle-assembly checkpoint is activated, MAD2 forms a ternary **complex** with CDC20 and APC to prevent activation of APC, and thereby arrests cells at prometaphase.

A Mad2 mutant containing serine to aspartic acid mutations mimicking the C-terminal phosphorylation events fails to **interact** with Mad1 or the APC/C and acts as a dominant-negative antagonist of wild-type Mad2.

We find HsMad1 associated with HsMad2.

Mad2 binding to Mad1 and Cdc20, rather than oligomerization, is required for the spindle checkpoint.

Mad2 also undergoes a similar striking structural change upon **binding** to a Mad1 or Cdc20 binding motif peptide.

A MHC-encoded ubiquitin-like protein (FAT10) **binds** noncovalently to the spindle assembly checkpoint protein MAD2.

The Mad2 spindle checkpoint protein undergoes similar major conformational changes upon **binding** to either Mad1 or Cdc20.

In this study, Mad2 and its **target** Slp1 were visualized in a tractable organism, fission yeast *Schizosaccharomyces pombe*.

We also show that Mad2 associates with the APC regulatory protein p55Cdc in mammalian cells as has been reported in yeast.

I show herein that in checkpoint-arrested cells, human Cdc20 forms two separate, inactive complexes, a lower affinity complex with Mad2 and a higher affinity **complex** with BubR1.

The Sequestration Model postulates that Mad2 and BubR1 bind and sequester Cdc20, an APC/C activator, away from APC/C so substrates whose destruction drives mitotic exit are no longer ubiquitinated.

Our observations support a model in which Nuf2 and Hec1 function to prevent microtubule-dependent stripping of Mad1 and Mad2 from kinetochores that have not yet formed stable kinetochore-microtubule attachments.

Reducing the levels of the checkpoint proteins BubR1 or Mad2 in human cancer cells or **inhibiting** BubR1 kinase activity provokes apoptotic cell death within six divisions except when cytokinesis is also inhibited.

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If you find iHOP useful please cite as "Hoffmann, R., Valencia, A. A gene network for navigating the literature. Nature Genetics 36: 664 (2004)".



Symbol	Name	Synonyms	Organism
Mad211	Mitotic spindle assembly checkpoint protein MAD2A	MAD2, Mad2a, MAD2-like 1	Mus musculus
UniProt	Q9J153, Q9Z1B5		
NCBI Gene	56150		
NCBI RefSeq	NP_062372		
NCBI Accession	AA09238, AAF69525, AF259902		
Homologues of Mad211 ... new			

More literature information for this gene ...

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Generation of Rb pathway lesions in normal and transformed cells **produces** aberrant Mad2 expression and mitotic defects leading to aneuploidy, such that elevated Mad2 contributes directly to these defects.

Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2.

The mouse homologue of Mad2 protein, which plays an important role in the M phase of the cell cycle, was revealed to associate with the box1/2 region specifically.

Mad2 protein was increased as a consequence of the absence of p53 function.

Mad2 is known to change affinity for binding partners cell cycle dependently.

Overexpression of Mad2 in meiosis I leads to a cell cycle arrest in metaphase I.

The increase in Mad2 protein was observed also at the cellular level by immunohistochemistry.

The Mad2 protein plays a significant role in accurate chromosome segregation in mitotic cells.

MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells.

In this report, we demonstrate that TCDD suppresses the expression of the checkpoint protein, Mad2.

MAD2 inhibits the anaphase-promoting complex when chromosomes are unattached to the mitotic spindle.

Dioxin suppresses the checkpoint protein, MAD2, by an aryl hydrocarbon receptor-independent pathway.

Deletion analysis indicated that interaction with box1/2 occurred through the C-terminal portion of Mad2.



Our data suggest that TCDD may increase chromosomal instability through the suppression of Mad2 expression.

Complete MAD2 inactivation has not been identified in human tumors, although partial defects are prevalent.



Differences in spindle association of the mitotic checkpoint protein Mad2 in mammalian spermatogenesis and oogenesis.  

The best producer among the several clones obtained was expanded and the secreted MAb (MAD2) purified and characterized.  

Complete loss of the tumor suppressor MAD2 causes premature cyclin B degradation and mitotic failure in human somatic cells.  

Concordantly, Mad2 is overexpressed in several tumour types, where it correlates with high E2F activity and poor patient prognosis.  

In contrast to the situation in spermatogenesis, Mad2 persisted at the kinetochores of normal, second-division oocytes at metaphase.  

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Furthermore, the mitotic checkpoint protein Mad2 is present throughout meiotic maturation and is recruited to unattached kinetochores.  



In addition, the association of Mad2 with second-division-metaphase kinetochores differed significantly in male versus female meiosis.  

Furthermore, Mad2^{+/-} mice develop lung tumours at high rates after long latencies, implicating defects in the mitotic checkpoint in tumorigenesis.  



This result indicates that loss of kinetochore-associated Mad2 is not essential for the metaphase-to-anaphase transition during the first meiotic division.  



Disruption of the male meiotic spindles with the microtubule depolymerizing agent nocodazole resulted in the appearance of Mad2 at nearly all kinetochores.  



In spermatogenesis, Mad2 remained at most kinetochores throughout the entire first meiotic division and was lost only at metaphase of the second meiotic division.  



We have investigated expression and subcellular localization of the spindle checkpoint protein Mad2 during rat and mouse spermatogenesis and in superovulated mouse oocytes.  

In contrast, the microtubule stabilizer taxol induced the loss of Mad2 from the majority of the first-division-metaphase kinetochores in which it was normally present in untreated cells.  

MAD2 and the panel of MAbs which are at present being purified may become a tool for studying the relevance of different domains of the anthracyclin molecule in terms of biologic activity.  

Our immunofluorescence studies demonstrate substantial differences in the localization patterns of kinetochore-associated Mad2 in these meiotic systems compared with previous studies of mitosis.  

Thus, anaphase-promoting complex substrates exhibit distinct sensitivities in the presence of different MAD2 doses, which in turn determine MAD2's role as either a tumor suppressor or an essential gene.  

MAD2 cross-reacts to varying degrees with anthracycline compounds such as some DXR analogues and derivatives, but does not recognize anthracene and anthraquinone structures, with the exception of weakly reacting Mitoxantrone.  

Expression of a dominant-negative Mad2 protein interferes with proper spindle checkpoint

arrest. **CONCLUSIONS:** Errors in meiosis I cause missegregation of chromosomes and can result in the generation of aneuploid embryos with severe birth defects.

Second, the absolute rate of chromosome missegregation may be increased by alterations in the levels of two proteins, separase and Mad2, which are important for maintaining chromosomal segregation and the normal spindle checkpoint during mitosis.

By employing RNA interference in human somatic cells, we found that severe reduction of MAD2 protein levels results in mitotic failure and extensive cell death arising from defective spindle formation, incomplete chromosome condensation, and premature mitotic exit leading to multinucleation.

In contrast, the postmitotic trophoblast giant cells survive without Mad2.

Cell cycle-dependent interaction of Mad2 with conserved Box1/2 region of human granulocyte-macrophage colony-stimulating factor receptor common betac.

Double labelling immunofluorescence with MAD-2 and a mouse monoclonal antibody against vimentin, the intermediate filament protein of cells of mesenchymal origin, showed coincidental staining which was distinct from that seen with antibodies against other cytoskeletal proteins.

At metaphase I and II, MC kinetochores assembled MAD2 and BUBR1 spindle checkpoint proteins.

These results show that the major tissue and leucocyte protein recognized by MAD-2 is vimentin.

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Using immunoblotting, both MAD-2 and anti-vimentin reacted with a 55 kDa tissue component, and with purified vimentin.

Indeed, in survivin-depleted cells, BubR1 and Mad2 are prematurely displaced from kinetochores, yet no tension is generated at kinetochores.

We show that the mitotic checkpoint protein Mad2 is a direct E2F target and, as a consequence, is aberrantly expressed in cells with Rb pathway defects.

Here we report that deletion of one MAD2 allele results in a defective mitotic checkpoint in both human cancer cells and murine primary embryonic fibroblasts.

Cells overexpressing Aurora-A inappropriately enter anaphase despite defective spindle formation, and the persistence of Mad2 at the kinetochores, marking continued activation of the spindle assembly checkpoint.

Northern analysis of two different spindle assembly checkpoint protein gene products from human, BUB1 and MAD2, reveals an expression pattern with common tissue distribution consistent with roles in a common pathway.

First, Western blot analysis demonstrated that the levels of two proteins involved in regulating sister chromatid separation and the spindle checkpoint, Mad2 and separase (ESPL1) were increased in null compared with WT cells.

The results of northern blot analysis for small proline-rich protein 2 (Spr2), 17beta-hydroxysteroid dehydrogenase type 2 (17betaHSD-2), high mobility group 2 (Hmg2), mitotic checkpoint component 2 (Mad2) and an EST AW555366 mRNA were consistent with that of microarray analysis.

Peptides corresponding to the box1 sequence also bound to Mad2, and mutation of the box1 decreased the Mad2 interaction.

Thus, mRev3 could be required to repair a form of externally induced DNA damage that otherwise accumulates during clonal expansion or, consistent with the high homology shared between its Rev7 partner and the mitotic checkpoint gene product Mad2 [6], mRev3 might play a role in cell proliferation and genomic stability even in the absence of environmentally induced damage.

To determine the function of the mitotic checkpoint protein Mad2 during normal cell division and when mitosis goes awry, we have knocked out Mad2 in mice.

Suppression of Mad2 was also observed in aryl hydrocarbon receptor-deficient mouse embryonic fibroblasts, suggesting that TCDD suppresses Mad2 by a novel TCDD receptor signaling mechanism.

Binding affinity of Mad2 to box1/2 increased in the late M phase, suggesting the possibility that GM-CSF participates in regulation of the M phase check point through interaction with Mad2.

Cenpag/g embryos assemble functional kinetochores that bind to a host of centromere-specific structural and mitotic spindle checkpoint proteins (Cenpc, BubR1, Mad2 and Zw10).

We find that E5.5 embryonic cells lacking Mad2, like mad2 yeast, grow normally but are unable to arrest in response to spindle disruption.

Cyclin B is degraded prematurely in the MAD2 short interfering RNA-treated cells but not in MAD2^{+/-} cells, suggesting an explanation for the spindle failure and mitotic catastrophe in the MAD2 knockdown cells.

Antisera to CENP-A, CENP-B, CENP-C, CENP-E, CENP-F, INCENP, CLIP-170, dynein, dynactin subunits p150 (Glued) and Arp1, MCAK, Tsg24, p55CDC, HZW10, HBUB1, HBUBR1, BUB3, MAD2, ERK1, 3F3/2, topoisomerase II and a murine HP1 homologue, M31, were used in immuno-fluorescence experiments in conjunction with FISH employing specific DNA probes to clearly identify neocentromeric DNA.

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Symbol	Name	Synonyms	Organism
CG17498	CG17498-PA		Drosophila melanogaster
NCBI Gene	38656		
NCBI Accession	AAF50740, AAL48090, AE003565		
Homologues of CG17498 ...	new		

Unfortunately no literature information has been found.

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 click here for an enhanced query to **Google™** or **PubMed** including the synonyms for

CG17498 ...

Please note, that literature information is available only for genes with the  or  icons.

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Experimental evidence for the association between CG17498 and CG32000 from Y2H.

Experimental evidence for the association between CG17498 and CG15576 from Y2H.

Experimental evidence for the association between CG17498 and CG13214 from Y2H.





Symbol Name
mdf-2

Synonyms
CELK04085,
Y69A2AR.30a,
Y69A2AR.30b

Organism
Caenorhabditis elegans

NCBI Gene 177046
NCBI Accession AAF63495, AAK68576, AAM15619
Homologues of mdf-2 ... **new**

More literature information for this gene ...

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Antibodies that recognize the **MDF-2** protein localize to nuclei of the **cleaving** embryo in a cell-cycle-dependent manner. **mdf-1**, a gene encoding a product that interacts with **MDF-2**, is required for cell-cycle arrest and proper chromosome segregation in premeiotic germ cells treated with **nocodazole**, a microtubule-depolymerizing agent.

Here we show that **mdf-1** and **mdf-2** are components of the spindle-assembly checkpoint in *Caenorhabditis elegans*, and are essential for the long-term survival and fertility of this organism.

During anoxia-induced suspended animation, embryos lacking functional **SAN-1** or a second spindle checkpoint component, **MDF-2**, failed to arrest the cell cycle, exhibited chromosome missegregation, and showed reduced viability.

Experimental evidence for the association between **mdf-2** and **1G243** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **2M633** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **1C653** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **5D165** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **1I177** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **tc1-2** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **csn-5** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **3K840** from **Y2H**.

Experimental evidence for the association between **mdf-2** and **5M843** from **Y2H**.

2

Experimental evidence for the association between mdf-2 and 1L214 from Y2H.



Experimental evidence for the association between mdf-2 and 5O926 from Y2H.



Experimental evidence for the association between mdf-2 and air-1 from Y2H.



Experimental evidence for the association between mdf-2 and 3J401 from Y2H.



Experimental evidence for the association between mdf-2 and 2I64 from Y2H.



Experimental evidence for the association between mdf-2 and cpr-4 from Y2H.



Experimental evidence for the association between mdf-2 and hum-5 from Y2H.



Experimental evidence for the association between mdf-2 and 2L733 from Y2H.



Experimental evidence for the association between mdf-2 and 1C869 from Y2H.



Experimental evidence for the association between mdf-2 and apt-2C from Y2H.



Experimental evidence for the association between mdf-2 and tbp-1 from Y2H.



Experimental evidence for the association between mdf-2 and 3E52 from Y2H.



Experimental evidence for the association between mdf-2 and 5N852 from Y2H.



Experimental evidence for the association between mdf-2 and 4E167 from Y2H.



If you find iHOP useful please cite as "Hoffmann, R., Valencia, A. A gene network for navigating the literature. Nature Genetics 36, 664 (2004)".



Symbol Name

At3g25980

NCBI Gene 822195

Homologues of At3g25980 ... **new**

Synonyms

MPE11.16

Organism

Arabidopsis thaliana

Unfortunately no literature information has been found.

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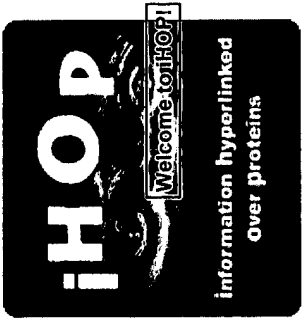
At3g25980 ...

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Symbol	Name	Synonyms	Organism
TUB1	Tubulin alpha-1 chain	YML085C	Saccharomyces cerevisiae
UniProt	P09733		
NCBI Gene	854889		
NCBI RefSeq	NP_013625.1		
NCBI Accession	AAA35180, CAA86653		
Homologues of TUB1 ... new			

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Filaments of cyano fluorescent protein-tagged **Fin1** **colocalize** with filaments of green fluorescent protein-tagged **Tub1** only in large-budded cells.

A systematic study of 51 **tub1** alleles suggests a correlation between specific failure to **interact** with **Bim1p** in the two-hybrid assay and synthetic lethality with the **bim1Delta** allele.

A previously uncharacterized yeast gene (**YER016w**) that we have named **BIM1** (**binding to microtubules**) was obtained from a two-hybrid screen of a yeast cDNA library using as bait the entire coding sequence of **TUB1** (encoding **alpha-tubulin**).

Aut7p is **attached** to microtubules via **Aut2p**, which interacts with tubulins **Tub1p** and **Tub2p**. **aut2-** and **aut7-** deleted cells are unable to deliver autophagic vesicles and the precursor of aminopeptidase I to the **vacuole**.

This arrest is due to lower levels of **alpha-tubulin**, a protein encoded by **TUB1** and **TUB3**, two intron-containing genes.

However, the **mcm19** null mutation conferred growth defects in the presence of a mutation in the **TUB1** gene coding for **alpha-tubulin**.



Tubulin was purified from wild-type and deletion strains lacking either **Tub1** or **Tub3**, and parameters of microtubule dynamics were examined.



This technique was used to tag **Cdc3** and **Tub1** with GFP, YFP and CFP, which were readily visualized by fluorescence microscopy and localized as expected.



Mutations that suppressed the cold-sensitive phenotypes of two of the **TUB1** alleles occurred in **TUB2**, the single structural gene specifying **beta-tubulin**.



Recent investigations have confirmed the presence of one **alpha-tubulin** gene (**TUB1**) and one **beta-tubulin** gene (**TUB2**) in the dimorphic fungus *Histoplasma capsulatum*.



The noncomplementation between **tub1** and **tub2** mutations is gene specific and allele specific, suggesting that the phenotype is due to an interaction at the protein level.



The yeast *Saccharomyces cerevisiae* has two alpha-tubulin genes, TUB1 and TUB3, either of which alone is sufficient for these processes when present in a high enough copy number.  



Genomic replacement of an intronless TUB1 gene relieves the benomyl sensitivity of prp17 mutants; however, they remain temperature sensitive, implying multiple limiting factors for mitosis.  


Nucleotide sequencing studies revealed that the two genes, named TUB1 and TUB3, encoded gene products of 447 and 445 amino acids, respectively, that are highly homologous to alpha-tubulins from other species.  



The systematic tub1 mutations were placed, along with the comparable set of tub2 mutations previously described, onto a model of the yeast alpha-beta-tubulin dimer based on the three-dimensional structure of bovine tubulin.  

The relative importance in these processes of the two divergent alpha-tubulin genes of the budding yeast *Saccharomyces cerevisiae*, TUB1 and TUB3, was examined through the construction of null mutations and by increasing their copy number on chromosomes and on plasmids.  

The yeast *Saccharomyces cerevisiae* has two genes for alpha-tubulin, TUB1 and TUB3, and one beta-tubulin gene, TUB2.  

Splicing of TUB1 and TUB3 transcripts, which encode alpha-tubulin, was analyzed in prp17 and other second-step factor mutants.  

Transformation of mutant cells with genomic libraries repeatedly identified three different suppressors: the two wild-type alpha-tubulin genes, TUB1 and TUB3; and BUB3.  

Genetic interactions between stu1 alleles and alleles of TUB1 and TUB2 suggest that Stu1p specifically interacts with microtubules.  

top

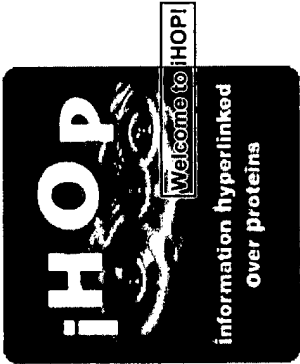
In order to ascertain associations of other tubulins with dinitroaniline resistance, four beta-tubulin cDNA classes (designated TUB1, TUB2, TUB3, and TUB4) were isolated from dinitroaniline-susceptible and -resistant biotypes.  

TUB1 splicing is inefficient in prp17, prp16 and prp22, and marginally affected in prp18, slu7-1 and psf1-1.  

Such mutants fall into six complementation groups: TUB1, TUB2 and TUB3, the three tubulin genes of yeast, and three new genes, which we have named CIN1, CIN2 and CIN4.  

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Symbol	Name	Synonyms	Organism
MAD2	Mitotic spindle checkpoint component MAD2	J1256, Mitotic MAD2 protein, YJL030W	Saccharomyces cerevisiae

UniProt P40958
NCBI Gene 853422
NCBI RefSeq NP_012504.1
NCBI Accession AA21385, CAA89321

Homologues of MAD2 ... **new**

More literature information for this gene  ...

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Comparisons of the amino acid sequences suggest that the proteins encoded by the Saccharomyces cerevisiae genes **MAD2** and **BET2** are the yeast counterparts of the mammalian Rab GG transferase alpha and beta subunits, respectively.



Overexpression of mph1, like overexpression of mad2, mimics activation of the checkpoint and imposes a metaphase arrest. mph1 protein shares sequence similarity with Mps1p, a dual specificity kinase that functions in the spindle checkpoint of the budding yeast Saccharomyces cerevisiae.



Bipolar orientation of chromosomes in Saccharomyces cerevisiae is monitored by **Mad1** and **Mad2**, but not by **Mad3**.



In contrast, we show that whereas Saccharomyces cerevisiae **Bub1p** and **Bub3p** are bound to kinetochores early in mitosis as part of the normal cell cycle, **Mad1p** and **Mad2p** are kinetochore bound only in the presence of spindle damage or kinetochore lesions that interfere with chromosome-microtubule attachment.



Bet2p forms a **complex** with **Mad2p** that appears to bind geranylgeranyl pyrophosphate, but not farnesyl pyrophosphate.



Budding yeast **Bub2** is localized at spindle pole bodies and **activates** the mitotic checkpoint via a different pathway from **Mad2**.



A MHC-encoded ubiquitin-like protein (**FAT10**) **binds** noncovalently to the spindle assembly checkpoint protein **MAD2**.



We also show that **Mad2** **associates** with the APC regulatory protein **p55Cdc** in mammalian cells as has been reported in yeast.



Yeast two-hybrid screening of a human lymphocyte library and immunoprecipitation studies revealed that **FAT10** noncovalently **associated** with **MAD2**, a protein implicated in a cell-cycle checkpoint for spindle

5

assembly during anaphase.

Genetic interactions with checkpoint and apc mutants suggest Cdc28 may **regulate** checkpoint arrest downstream of the MAD2 and BUB2 pathways.

On the basis of these results and genetic analysis of double mutants, we propose a model in which Mad1p bound to a Nup53p-containing **complex** sequesters Mad2p at the NPC until its release by activation of the spindle checkpoint.

The Cdc14 liberation from the nucleolus was **inhibited** by the Mad2 checkpoint and by the Bub2 checkpoint in a different manner when microtubule organization was disrupted.

Mad1p forms a tight **complex** with Mad2p [7], and has been shown to recruit Mad2p to kinetochores [8].

Analysis of the genetic interaction of BFA1 with known mitotic checkpoint genes suggest that Bfa1 **functions** in the same pathway with Bub2 but not with Mad1 or Mad2.

In addition, we find that Mad3p **interacts** with Mad2p and the cell cycle regulator Cdc20p.

MAD3 encodes a novel component of the spindle checkpoint which **interacts** with Bub3p, Cdc20p, and Mad2p.

The following observations indicate that Bub2 and Mad1, 2 probably activate the checkpoint via different pathways: (a) unlike the other Mad and Bub proteins, Bub2 localizes at the spindle pole body (SPB) throughout the cell cycle; (b) the **effect** of concomitant lack of Mad1 or Mad2 and Bub2 is additive, since nocodazole-treated mad1 bub2 and mad2 bub2 double mutants rereplicate DNA more rapidly and efficiently than either single mutant; (c) cell cycle progression of bub2 cells in the presence of nocodazole requires the Cdc26 APC subunit, which, conversely, is not required for mad2 cells in the same conditions.

In addition, Mad2p **binds** to all of the different **phosphorylated** isoforms of Mad1p that can be resolved on SDS-PAGE.

Checkpoint proteins Mad2p and Mad3p/BubR1p **bind** and **inhibit** Cdc20p, an activator for the anaphase-promoting complex (APC).

In extracts prepared from M phase, but not from interphase HeLa cells, p55CDC coimmunoprecipitates with three important elements of the M phase checkpoint machinery: Cdc27, Cdc16, and Mad2. p55CDC is required for **binding** Mad2 with the Cdc27 and Cdc16.

The spindle checkpoint of budding yeast depends on a tight complex between the Mad1 and Mad2 proteins.

Therefore MAD2 and BET2 gene products may physically interact to form a geranylgeranyl transferase complex.

Studies in yeast have shown that binding of MAD1 to MAD2 is important for the checkpoint function of the latter.

RAM2 is also homologous to MAD2, a yeast gene whose product has been implicated in the feedback control of mitosis.

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MAD2beta has 23% sequence identity with MAD2, which is a component of the spindle assembly (or mitotic) checkpoint mechanism.

In the same screen which led to the isolation of mad2 and mph1, we also isolated dph1, a cDNA that encodes a protein 46% identical to an *S. cerevisiae* SPB duplication protein, Dsk2p.

In this context, the mitotic arrest deficiency protein 2 (MAD2) censors chromosomal mis-segregation by monitoring microtubule attachment/tension, a role that requires its attachment to kinetochores.

We have identified mitotic arrest deficient 2 (MAD2) as a binding partner of the TACE cytoplasmic domain, and a novel MAD2-related protein, MAD2beta, as a binding partner of the MDC9 cytoplasmic domain.

This arrest depends on the spindle checkpoint protein Mad2.

The 572 del A mutation creates a truncated MAD2 protein product.

Mad2 transiently associates with an APC/p55Cdc complex during mitosis.

The mitotic arrest defects of ibd2Delta were not recovered by MAD2, or vice versa.

We cloned MAD2, which encodes a putative calcium-binding protein whose disruption is lethal.

The kinetochore is an integral component of the MAD2 branch of the spindle checkpoint pathway.

Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase.

We support the idea that MAD2 is attachment-sensitive and that tension stabilizes microtubule attachments.

Most current models of spindle assembly checkpoint signaling involve inhibition of the Cdc20-APC by Mad2 protein.

We also found that the delay in mitotic exit in mutants with misoriented spindles depended on BUB2 and BFA1, but not on MAD2.

We have cloned the MAD2 gene, which encodes a protein of 196 amino acids that remains at a constant level during the cell cycle.

These studies suggest that Mad2 protein function is essential for the timing of anaphase onset in somatic cells at each mitosis.

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Further, the tension-sensitive 3F3/2 phosphopeptide colocalized, and was lost concomitantly, with MAD2 staining at the meiotic kinetochore.

Interestingly, we found that in the yeast two-hybrid system, the interaction of F2 and MAD2 was stronger than that of intact p55CDC/hCDC20.



We tested whether the Mad2 protein participates in regulating the timing of anaphase onset in mammalian cells in the absence of microtubule drugs.

Two recent papers report the cloning of human and *Xenopus* homologues of one of these yeast genes, called MAD2 (for mitotic arrest deficient-2)(1,2).


The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns.  



Interaction of MAD2 with the carboxyl terminus of the insulin_receptor but not with the IGFIR. Evidence for release from the insulin_receptor after activation.  

Introduction of antibodies to the MAD2 protein into living mammalian cells or Xenopus egg extracts abrogates the M phase arrest induced by microtubule inhibitors.  



In yeast, the Mad2 protein is required for the M phase arrest induced by microtubule inhibitors, but the protein is not essential under normal culture conditions.  



Finally, T47D, a human breast tumor cell line that is sensitive to taxol and nocodazole, had reduced MAD2 expression and failed to arrest in mitosis after nocodazole treatment.  


MAD2 staining was primarily observed on mitotic kinetochores that lacked attached microtubules; i.e., at prometaphase or when the microtubules were depolymerized with oryzalin.  



Unattached kinetochores are believed to release an activated form of Mad2 that inhibits APC/C-dependent ubiquitination and subsequent proteolysis of components needed for anaphase onset.  

These results suggest a model for the regulation of the APC by Mad2 and may explain how the spindle assembly checkpoint apparatus controls the timing of mitosis under normal growth conditions.  



When this condition is not met, MAD2, a component of the spindle checkpoint complex, associates with p55CDC/hCDC20 to inhibit ubiquitination of substrates by the anaphase-promoting complex (APC).  

Overexpression of BUB1, which interacts genetically with BUB3 and which is involved in the same checkpoint pathway, also rescues the cold sensitivity of tub1-729, but another checkpoint gene, MAD2, does not.  



In contrast, the loss of MAD2 staining in meiosis was not correlated with initial microtubule attachment but was correlated with a measure of tension: the distance between homologous or sister kinetochores (in meiosis I and II, respectively).  

Immunofluorescence studies reveal that cells blocked in metaphase by chromosome damage contain one or more Mad2-positive kinetochores, and the block is rapidly overridden when the cells are microinjected with a dominant-negative construct of Mad2 (Mad2deltaC).  



Thus, it is likely that p55CDC mediates the association of Mad2 with the cyclosome/anaphase-promoting complex.  

Bet2p and Mad2p are components of a prenyltransferase that adds geranylgeranyl onto Ypt1p and Sec4p [see comments].  

We have also found that the MAD2 but not the BUB2 spindle checkpoint gene is required for efficient arrest of yku70Delta mutants.  

One pathway, defined by MAD2, controls the metaphase-to-anaphase transition and the other, defined by BUB2, controls the exit from mitosis [3-6].  

top

The MAD2 branch of the pathway responds to kinetochore microtubule interactions and the BUB2 branch  

of the pathway operates within the cytoplasm, responding to spindle misorientation.

The second one, that requires FZY function (even in the absence of MAD2 protein and when the spindle assembly checkpoint is not activated) is not yet understood at its molecular level.

Mammalian p55CDC mediates association of the spindle checkpoint protein Mad2 with the cyclosome/anaphase-promoting complex, and is involved in regulating anaphase onset and late mitotic events.

The yeast MAD2 protein was first identified in a genetic screen to identify cell cycle checkpoint regulatory proteins, yet the mechanism by which MAD2 functions in cell cycle control is currently unclear.

Here we isolate a new temperature-sensitive mad2 mutant, mad2-2ts, and find that Mad2p is required for the membrane association of Ypt1p and Sec4p, two prenylated small GTP-binding proteins involved in protein trafficking.

Evidence for an interaction of the metalloprotease-disintegrin tumour necrosis factor alpha convertase (TACE) with mitotic arrest deficient 2 (MAD2), and of the metalloprotease-disintegrin MDC9 with a novel MAD2-related protein, MAD2beta.

The independent identification of MAD2 and MAD2beta as potential interacting partners of distinct metalloprotease-disintegrins raises the possibility of a link between metalloprotease-disintegrins and the cell cycle, or of functions for MAD2 and MAD2beta that are not related to cell cycle control.

In contrast, the postmitotic trophoblast giant cells survive without Mad2.

Blocking APC^{Cdh1}-mediated Clb2 proteolysis and chromosome re-duplication does not require Mad2 but a different protein, Bub2.

Gel filtration and co-immunoprecipitation analyses reveal that Mad2p tightly associates with another spindle checkpoint component, Mad1p.

The normal centromere has been deleted and a neocentromere containing CENP-A, -C, -E and Mad2 but not CENP-B has formed close to the breakpoint.

We show that two proteins required for the execution of the spindle checkpoint, Mad1p and Mad2p, reside predominantly at the NPC throughout the cell cycle.

We show that spindle damage blocks sister chromatid separation solely by inhibiting APC^{Cdc20}-dependent Pds1 proteolysis and that this process requires Mad2.

Moreover, although Mad1p and Mad2p perform essential mitotic functions during every division cycle in mammalian cells, they are required in budding yeast only when mitosis goes awry.

In the present report, we demonstrate that the inhibitory association between Mad2 and the APC component Cdc27 also takes place transiently during the early stages of a normal mitosis and is lost before mitotic exit.

Hierarchical clustering analysis of the set1(-) expression profile revealed a correspondence to that of a mad2(-) strain, suggesting that the transcriptional defect in the set1(-) strain may be due to changes in chromatin structure.

Here we show that in budding yeast, BUB2 and BFA1 are also required for the maintenance of G2/M



arrest in response to DNA damage and to spindle misorientation. cdc13-1 bub2 and cdc13-1 bfa1 but not cdc13-1 mad2 double mutants rebud and reduplicate their DNA at the restrictive temperature.

We identified a human cDNA encoding a protein that appears to be the human homolog of the yeast MAD2 protein, which we term hMAD2.

Since high levels of Mad2 overcome checkpoint loss in Mps1-depleted extracts, Mps1 acts upstream of Mad2-mediated inhibition of APC/C.

The checkpoint response, however, is significantly weakened in Sgt1-depleted cells, and this correlates with a dramatic reduction in kinetochore levels of Mad1, Mad2 and BubR1.

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Northern analysis of two different spindle assembly checkpoint protein gene products from human, BUB1 and MAD2, reveals an expression pattern with common tissue distribution consistent with roles in a common pathway.

Human, or Homo sapiens, MAD2 (hsMAD2) was localized at the kinetochore after chromosome condensation but was no longer observed at the kinetochore in metaphase, suggesting that MAD2 might monitor the completeness of the spindle-kinetochore attachment.

We find that three genes (MAD2, BUB1, and BUB2) that are required for the spindle assembly checkpoint in budding yeast (defined by antimicrotubule drug-induced arrest or delay) are also required in the establishment and/or maintenance of kinetochore-induced delays.

Like Bet2p and Mad2p, Mrs6p is required for the membrane attachment of Ypt1p and Sec4p in vivo.

Checkpoint arrest in the absence of bipolar orientation and tension (induced by replication block in a cdc6 mutant) was lacking in cells without MAD1 or MAD2.

Deletion and mutational analysis of both proteins indicate that association of Mad2p with Mad1p is critical for checkpoint function and for hyperphosphorylation of Mad1p.

Whereas the MAD2 and BUB1 genes were absolutely required for delay, loss of BUB2 function resulted in a partial delay defect, and we suggest that BUB2 is required for delay maintenance.

Combined immunolocalization of MAD2 and a recently cloned maize CENPC homologue indicates that MAD2 localizes to an outer domain of the prometaphase kinetochore.

In budding yeast, Mad1, Mad2, and Mad3 proteins are equally required for arrest in the presence of damage induced by antimicrotubule drugs or catastrophic loss of spindle structure.

Mps1 is essential for the checkpoint because it is required for recruitment and retention of active CENP-E at kinetochores, which in turn is necessary for kinetochore association of Mad1 and Mad2.

On activation of the spindle checkpoint, we detect changes in the interactions between these proteins, including the release of Mad2p (but not Mad1p) from the NPC and the accumulation of Mad2p at kinetochores.

Thus, mRev3 could be required to repair a form of externally induced DNA damage that otherwise accumulates during clonal expansion or, consistent with the high homology shared between its Rev7 partner and the mitotic checkpoint gene product Mad2 [6], mRev3 might play a role in cell proliferation and

genomic stability even in the absence of environmentally induced damage.

Mad2 and Mad3 coprecipitated with Cdc20 at all stages of the cell cycle.

We conclude that Mad1 and Mad2 are required to detect bipolar orientation and/or tension at kinetochores, whereas Mad3 is not.

Therefore, mitotic arrest induced by excess Mps1p expression is due to the action of the MAD2 branch of the spindle checkpoint pathway and excess Mps1p acts downstream of the kinetochore.

We determined which spindle defects the checkpoint can detect by examining the interaction of mutations that compromise the checkpoint (mad1, mad2, and mad3) with those that damage various structural components of the spindle.

To determine the function of the mitotic checkpoint protein Mad2 during normal cell division and when mitosis goes awry, we have knocked out Mad2 in mice.

CDH1 showed genetic interactions with MAD2 and PDS1, genes encoding components of the mitotic spindle assembly checkpoint that acts at metaphase to prevent premature chromosome segregation.

We show that low concentrations of nocodazole delay cell division under the control of the previously identified mitotic checkpoint genes BUB1, BUB3, MAD1, and MAD2 and independently of BUB2.

Analysis of Mad1p phosphorylation in other spindle assembly checkpoint mutants reveals that this response to microtubule-disrupting agents is defective in some (mad2, bub1, and bub3) but not all (mad3, bub2) mutant strains.

We find that formation of this complex requires Mad2p and Mps1p but not Mad3p or Bub2p.

In the two-hybrid system, three proteins that are components of the checkpoint, Mad1, Mad2, and Mad3, were shown to interact with Cdc20, a protein required for exit from mitosis.

Our data imply that Mad1, Mad2, Mad3 and Bub1 regulate APCCdc20, whereas Bub2 regulates APCCdh1.

We find that E5.5 embryonic cells lacking Mad2, like mad2 yeast, grow normally but are unable to arrest in response to spindle disruption.

This process requires the association between Cdc20p and Mad2p, and functional APC, but is independent of the known destruction boxes in Cdc20p and the other APC activator Cdh1p.

We have examined the status of proteins associated with the checkpoint protein complex (BUB1, BUBR1, BUB3, MAD2), the anaphase-promoting complex (Tsg24, p55CDC), and other proteins associated with mitotic checkpoint control (ERK1, 3F3/2 epitope, hZW10), on a human dicentric chromosome.

The pattern of kinetochore localization of hMPS1 in CENP-E defective cells suggests that their interaction with the kinetochore is sensitive to microtubule occupancy rather than kinetochore tension. hMPS1 is required for MAD1, MAD2 but not hBUB1, hBUBR1 and hROD to bind to kinetochores.

The binding of Mad2 depended on Mad1 and that of Mad3 on Mad1 and Mad2.

Similar to cells overexpressing MPS1, the BUB1-5 delay was dependent upon the functions of the other



top



checkpoint genes, including **BUB2** and **BUB3** and **MAD1**, **MAD2**, and **MAD3**.

The cold sensitivity of tub1-729 is suppressed by extra copies of a subset of the mitotic checkpoint genes **BUB1**, **BUB3**, and **MPS1**, but not **MAD1**, **MAD2**, and **MAD3**.

Experimental evidence for the association between **MAD2** and **MGS1** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **EBS1** from **Y2H**.

Experimental evidence for the association between **MAD2** and **NUP157** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **SAP4** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **SIN4** from **IntAct & Y2H**.

Experimental evidence for the association between **MAD2** and **GLO3** from **IntAct**.

Experimental evidence for the association between **MAD2** and **AHA1** from **Y2H**.

Experimental evidence for the association between **MAD2** and **MSB2** from **IntAct & Y2H**.



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THIRD EDITION

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homologous chromosome (homologue)

One of two copies of a particular chromosome in a diploid cell, each copy being derived from a different parent.

homology

Similarity in structure of an organ or a molecule, reflecting a common evolutionary origin. Specifically, such a similarity in protein or nucleic acid sequence. Contrasted with analogy—a similarity that does not reflect a common evolutionary origin.

homozygote

Diploid cell or organism having two identical alleles of a specified gene.

housekeeping gene

Gene serving a function required in all the cell types of an organism, regardless of their specialized role.

hybridization

Process whereby two complementary nucleic acid strands form a double helix during an annealing period; a powerful technique for detecting specific nucleotide sequences.

hybridoma

Cell line used in the production of monoclonal antibodies; obtained by fusing antibody-secreting B lymphocytes with cells of a lymphocyte tumor.

hydrocarbon

Compound that has only carbon and hydrogen atoms.

hydrolysis (adjective hydrolytic)

Cleavage of a covalent bond with accompanying addition of water, —H being added to one product of the cleavage and —OH to the other.

hydrophilic

Polar molecule or part of a molecule that forms enough hydrogen bonds to water to dissolve readily in water. (Literally, "water loving.")

hydrophobic (lipophilic)

Nonpolar molecule or part of a molecule that cannot form favorable bonding interactions with water molecules and therefore does not dissolve in water. (Literally, "water hating.")

hydroxyl (—OH)

Chemical group consisting of a hydrogen atom linked to an oxygen, as in an alcohol.

hypertonic

Describes any medium with a sufficiently high concentration of solutes to cause water to move out of a cell due to osmosis. (From Greek *huper*, over.)

hypotonic

Describes any medium with a sufficiently low concentration of solutes to cause water to move into a cell due to osmosis. (From Greek *hupo*, under.)

immortalization

Production of a cell line capable of an unlimited number of cell divisions. Can be the result of a chemical or viral transformation or of fusion with cells of a tumor line.

immune response

Response made by the immune system of a vertebrate when a foreign substance or microorganism enters its body.

immune system

Population of lymphocytes and other white blood cells in the vertebrate body that defends it against infection.

immunoglobulin (Ig)

An antibody molecule. Higher vertebrates have five classes of immunoglobulin—IgA, IgD, IgE, IgG, and IgM—each with a different role in the immune response.

immunoglobulin like (Ig-like) domain

Characteristic protein domain of about 100 amino acids that is found in antibody molecules and in many other proteins that form the Ig superfamily.

in situ hybridization

Technique in which a single-stranded RNA or DNA probe is used to locate a gene or an mRNA molecule in a cell or tissue. (See also **hybridization**.)

in vitro

Term used by biochemists to describe a process taking place in an isolated cell-free extract. Also used by cell biologists to refer to cells growing in culture (*in vitro*), as opposed to in an organism (*in vivo*). (Latin for "in glass.")

in vivo

In an intact cell or organism. (Latin for "in life.")

induction (embryonic)

Change in the developmental fate of one tissue caused by an interaction with another tissue.

inflammatory response

Local response of a tissue to injury or infection. Caused by invasion of white blood cells, which release various local mediators such as histamine.

initiation factor

Protein that promotes the proper association of ribosomes with mRNA and is required for the initiation of protein synthesis.

inositol

Cyclic molecule with six hydroxyl groups that forms the hydrophilic head group of inositol phospholipids.

inositol phospholipids (phosphoinositides)

One of a family of lipids containing phosphorylated inositol derivatives. Although minor components of the plasma membrane, they are important in signal transduction in eucaryotic cells. (See Figure 15–29.)

insulin

Polypeptide hormone that is secreted by β cells in the pancreas and helps regulate glucose metabolism in animals.

integrin

Member of the large family of transmembrane proteins involved in the adhesion of cells to the extracellular matrix.

interleukin

Secreted peptide or protein that mainly mediates local interactions between white blood cells (leucocytes).

intermediate filament

Fibrous protein filament (about 10 nm in diameter) that forms ropelike networks in animal cells. One of the three most prominent types of cytoskeletal filaments.

internal membrane

Eucaryotic cell membrane other than the plasma membrane. The membranes of the endoplasmic reticulum and the Golgi apparatus are examples.